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SUMMARY OF DOCTORAL THESIS

Name: Fatma Fakhry Abdel-Motaal

Title: THE ROLE OF SECONDARY METABOLITES OF THE MEDICINAL SOLANACEOUS PLANT (*HYOSCYAMUS MUTICUS* L.) AND ITS ASSOCIATED FUNGI IN PLANT-FUNGAL INTERACTIONS

(薬用植物 *Hyoscyamus muticus* L. と菌の相互作用における二次代謝物および共存菌の役割)

Hyoscyamus muticus (Egyptian henbane), which belongs to the family solanaceae, is widely distributed in the Egyptian deserts and has been known to produce useful secondary metabolites. In general, secondary metabolites in plants are natural products referring to the environmental condition surrounded the plants such as those important for defense against attacking organisms especially microbes or those attracting their beneficial organisms. The secondary metabolites produced most abundantly by *H. muticus* are the tropane alkaloids, hyoscyamine and scopolamine. In addition, desert plants communicate with other organisms to survive under the hard climatic conditions including high temperature and extreme dryness. The communication includes utilization of microbial activities to get the important nutrient or to increase the soil fertility.

In the present study, mycoflora of *H. muticus* grown in four different locations in Egyptian southern desert (Aswan region), Aswan university campus, Wadi Allaqi down stream part, Aswan airport road, and Sahari city, was described for the first time (Chapter 2). Eighty-one species and two varieties belonging to 31 genera were isolated from soils surrounding *H. muticus* plants, the surface of the plants, and inside the plants as endophytic fungi. *Aspergillus* was the most common genus in all study areas. A higher number of fungal species were isolated from rhizosphere soil and rhizoplane than from non-rhizosphere and other plant organs. Endophytic fungi were also isolated from all plant parts of *H. muticus.*

Despite the fact that *H. muticus* plants contain significant amounts of antifungal compounds including scopolamine and hyoscyamine, a variety of fungi can colonize the plants, suggesting a good communication between *H. muticus* and the fungi under desert conditions. To verify this hypothesis, responses of *H. mutics*-associated fungi to hyoscyamine and scopolamine and effects of plant extracts on the growth of those fungi and other pathogenic fungi of important crops were examined using TLC-bioautography (Chapter 3). All fungal strains (40 strains) examined were tolerant to scopolamine and sensitive to hyoscyamine, exhibiting a growth inhibition zone around the hyoscyamine spot on the bioautography plate. Fungal strains were grouped into three types based on the appearance of the inhibition zone: (i) 17 strains exhibiting a clear inhibition zone, which remained clear at 8 d after incubation (type I); (ii) 22 strains exhibiting the inhibition zone with a brown circle surrounding the zone and regrowth within the inhibition zone (type II); (iii) one strain exhibiting the inhibition zone with no brown circle and regrowth within the inhibition zone (type III). In the type II and type III strains, hyoscyamine disappeared, and other alkaloids were found in the inhibition zones in its place. Hyoscyamine-feeding experiments using *Penicillium purpurogenum* (type II) and *Cunninghamella elegans* (type III) revealed that these fungi may convert hyoscyamine to a new alkaloid compound, which would be less toxice to survive in the symbiotic association with *H. muticus* plant. Moreover, Plant extracts with chloroform (alkaloids) and *n*-butanol (non-alkaloids) exhibited antifungal activities, suggesting that antifungal compounds other than alkaloids, which were supposed to be saponins, were also produced by *H. muticus* as secondary metabolites.

Antifungal activity of hyoscyamine against two rice pathogens, *Magnaporthe oryzae* and *Rhizoctonia solani*, was studied in detail (Chapter 4). The minimum inhibitory concentration of hyoscyamine that resulted in distinctive inhibition (MIC50) was 1 μg/ml for both fungi. Exposure to hyoscyamine caused the leakage of electrolytes from the mycelia of both fungi. Hyoscyamine (>1μg/ml) irreversibly delayed or inhibited conidial germination and appressorium formation in *M. oryzae* grown on polystyrene plates. Hyoscyamine effectively inhibited the attachment of conidia to the surface of the rice (*Oryza sativa*) leaves and inhibited appressorium formation on the leaves. A high concentration of scopolamine (1000 μg/ml) also delayed or inhibited conidial germination in *M. oryzae*, but conidial germination could be restored by washing the conidia with water. Antifungal activity of hyoscyamine was reduced by scopolamine. Rice leaves treated with hyoscyamine showed significantly suppression of *M. oryzae* infection in the inoculation experiments by more than 90% using 10 μg/ml hyoscyamine. Moreover the same concentration of hyoscyamine significantly reduced the disease index of sheath blight to ≤0.2 comparing with the control plant disease index which recorded >7.0. Hyoscyamine (>20 μg/ml) completely inhibited sclerotial germination and development in *R. solani*. These results suggest the potential use of hyoscyamine in controlling the blast and sheath blight diseases affecting rice and in studying the mechanisms that regulate conidial germination in *M. oryzae* and sclerotial germination and development in *R. solani*.

Antifungal activity of the endophytes isolated from *H. muticus* was also studied (Chapter 5). A total of 44 strains of endophytic fungi isolated from the plant have been identified. Ten of these strains were inoculated into aseptic seedlings of *H. muticus*. Of the inoculated strains, nine were reisolated from the plants as endophytes. Using dual culture assay, we investigated whether eight of these strains, *Alternaria alternata*, *Aspergillus fumigatus*, *Drechslera hawaiiensis*, *Fusarium solani*, *Penicillium citrinum*, *Neoscytalidium dimidiatum*, *Thyrostromella myriana*, and *Ulocladium chartarum*, have antagonistic activities against two strains of plant pathogenic fungi, *Gibberella zeae* and *Thanatephorus cucumeris*, as well as six strains of non-pathogenic fungi, *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladorrhinum foecundissimum*, *Curvularia clavata*, *Penicillium janthinellum*, and *Ulocladium chartarum*. The endophytic fungi showed antagonistic activities against all the examined fungal strains. Extra- and intracellular fractions of *P. citrinum* and *N. dimidiatum* cultures, both of which showed high antagonistic activities, were extracted with ethyl acetate, *n*-butanol, and water. The resulting fractions were examined for their antifungal activity by performing bioautography coupled with thin-layer chromatography (TLC-bioautography). Extracellular fluids secreted by the cultures of these organisms showed high antifungal activities against fungi, including the two plant pathogenic fungi; this result indicates that these endophytes secrete antifungal compounds extracellularly.

In recent years, special focus was made on endophytic yeasts, most of which exhibited antimicrobial activities and industrial importance in different fields. In the present study, we isolated seven different species, which were classified to two groups, basidiomyceteous and ascomyceteous (Chapter 6). Four novel species of *Pseudozyma* belonging to basidiomyceteous group while two known species, *Pichia guilliermondii* and *Debaryomyces hansenii*. Identification of all yeast species was depending on morphological, physiological and Phylogentics characteristics (Chapter 7).

Two fungi, which cause new diseases on *H. muticus*, were identified in the present study. The identification of both fungi was depend on the morphologyical characteristics and nucleotide sequences of the internal transcribed spacer region of ribosomal DNA (rDNA-ITS) and the D1/D2 region of 28S rDNA. The first fungus is *Cladosporium herbarum* which causes severe brown spots on the leaf (Chapter 8). The symptoms started as leaf lesions under relatively humid conditions (>70%) and at slightly high temperatures (20–30°C). The disease was characterized by circular distinct patches of white spots that turned brown upon sporulation of the fungus. The spots increased in size over time, which led to leaf curling and defoliation. The disease name was proposed as *Cladosporium* leaf spot (Hanten-byo in Japanese) of Egyptian henbane for this new disease. Another disease caused by *Choanephora cucurbitarum* which first attach the blossoms and developing to the flower stalk, stem and leaves causing a floral rot (Chapter 9). Fungal sporulation appears as species with dark head on the infected tissue surface. The name Egyptian henbane floral rot disease was proposed.